201-14994

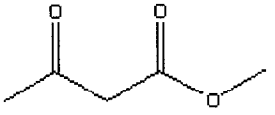
I. General Information

CAS Number: 105-45-3

Name: Methyl acetoacetate
Butanoic acid, 3-oxo-, methyl ester
Methyl 3-oxobutyrate
3-Oxobutanoic acid methyl ester
Acetoacetate, methyl
Acetoacetic acid, methyl ester
Methyl 3-oxobutanoate
Methyl acetylacetate

Formula : $C_5H_8O_3$

Structure:



II. Physical-Chemical Data

A. Melting Point

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: Not specified
Method	
Method:	Not specified
GLP:	Unknown
Year:	1968
Remarks:	
Results	
Melting Point value:	241.25 °K (- 32°C)
Decomposition:	
Sublimation:	
Remarks:	Fusion Point
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions.
Remarks:	
References:	*NIST Chemistry WebBook*, NIST Standard Reference Database 69 – March 2003 Release.

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: Not specified
Method	
Method:	Not specified
GLP:	Unknown
Year:	1989
Remarks:	
Results	
Melting Point value:	- 80 °C
Decomposition:	
Sublimation:	
Remarks:	
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions.
Remarks:	
References:	Budavari, S. (ed.), The Merck Index – Encyclopedia of Chemicals, Drugs and Biologicals, Rahway, NJ, Merck and Co., Inc., 1989.

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: ≥ 99 % (by GC)
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unknown
Remarks:	
Results	
Melting Point value:	- 28.0 °C
Decomposition:	
Sublimation:	
Remarks:	
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions.
Remarks:	
References:	Product Information Sheet for Methyl acetoacetate, Lonza Fine Organic Chemicals, Lonza AG, Visp, Switerland; August 1994 (Revised: November 10, 1999)

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: : > 99 % (by GC)
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unknown
Remarks:	
Results	
Melting Point value:	- 55.0 °C
Decomposition:	
Sublimation:	
Remarks:	Freezing Point
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions.
Remarks:	
References:	Material Safety Data Sheet for Methyl acetoacetate, Eastman Chemical Company, Kingsport, Tennessee; Revision Date: November 13, 2000

B. Boiling Point

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: Not specified
Method	
Method:	Not specified
GLP:	Unknown
Year:	1991-1992
Remarks:	
Results	
Boiling Point value:	171.7 °C
Pressure with units:	
Decomposition:	
Remarks:	.
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	
References:	Lide, D.R. (ed.), CRC Handbook of Chemistry and Physics, 72nd ed., Boca Raton, FL, CRC Press, 1991-1992

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: ≥ 99 % (by GC)
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unkown
Remarks:	
Results	
Boiling Point value:	170 °C
Pressure with units:	1,013 hPa
Decomposition:	
Remarks:	
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	
References:	Product Information Sheet for Methyl acetoacetate, Lonza Fine Organic Chemicals, Lonza AG, Visp, Switerland; January 1990 (Revised: November 10, 1999)

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: : ≥ 99 % (by GC)
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unkown
Remarks:	
Results	
Boiling Point value:	170 °C
Pressure with units:	Unknown
Decomposition:	Boils with decomposition
Remarks:	
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	
References:	Material Safety Data Sheet for Methyl acetoacetate, Eastman Chemical Company, Kingsport, Tennessee; Revision Date: November 13, 2000

C. Vapor Pressure

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: Not specified
Method	
Method:	Not specified
GLP:	Unknown
Year:	1963
Remarks:	
Results	
Vapor pressure value:	0.7 mm Hg
Temperatures:	20 °C
Decomposition:	
Remarks:	
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	
References:	Patty, F. (ed.), Industrial Hygiene and Toxicology, Volume II, Toxicology, 2nd ed., New York, Interscience Publishers, 1963.

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: Not specified
Method	
Method:	Not specified
GLP:	Unknown
Year:	1989
Remarks:	
Results	
Vapor pressure value:	0.892 mm Hg
Temperatures:	25 °C
Decomposition:	
Remarks:	
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	
References:	T.E.Daubert and Danner, R.P. (ed.), Physical and Thermodynamic Properties of Pure Chemicals, Design Institute for Physical Property Data, American Institute of Chemical Engineers, New York, Hemisphere Publication Corp. (1989)

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: ≥ 99 % (by GC)
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unknown
Remarks:	
Results	
Vapor pressure value:	Ca. 1 hPa
Temperatures:	20 °C
Decomposition:	
Remarks:	
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	
References:	Product Information Sheet for Methyl acetoacetate, Lonza Fine Organic Chemicals, Lonza AG, Visp, Switerland; October 1978

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: : > 99 % (by GC)
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unknown
Remarks:	
Results	
Vapor pressure value:	1.32 hPa
Temperatures:	20 °C
Decomposition:	
Remarks:	
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	
References:	Material Safety Data Sheet for Methyl acetoacetate, Eastman Chemical Company, Kingsport, Tennessee; Revision Date: November 13, 2000

D. Partition Coefficient

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	
Method	
Method:	Estimation
Remarks:	
Results	
Log P_{ow}:	- 0.69
Remarks:	
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	Estimated value based upon acceptable model.
References:	KOWIN, v 2.0b; Meylan, Wm. & Howard, P.H.(1996). User's Guide for Estimation Programs Interface (EPI), Syracuse Research Corporation, Syracuse, New York 13210.

E. Water Solubility

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity unknown
Method	
Method:	Unknown
Remarks:	
Results	
Value:	38 g/100 ml
Temperature:	
Description:	
Remarks:	
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	
References:	Patty, F. (ed.), Industrial Hygiene and Toxicology, Volume II, Toxicology, 2nd ed., New York, Interscience Publishers, 1963.

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity unknown
Method	
Method:	Unknown
Remarks:	
Results	
Value:	460.0 g/l
Temperature:	20 °C
Description:	
Remarks:	
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	
References:	Material Safety data sheet for Methyl acetoacetate, Hoechst AG, Hoechst, Germany; Issue Date: June 1988

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: : ≥ 99 % (by GC)
Method	
Method:	Unknown
Remarks:	
Results	
Value:	380.0 g/l
Temperature:	
Description:	
Remarks:	
Conclusions	
Data Quality	Reliable with restrictions
Reliability:	
Remarks:	
References:	Material Safety Data Sheet for Methyl acetoacetate, Eastman Chemical Company, Kingsport, Tennessee; Revision Date: November 13, 2000

III. Environmental Fate Endpoint

A. Photodegradation

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	
Method	
Method:	Estimation
Test type:	Atmospheric Oxidation
Remarks:	
Results	
Temperature:	25 °C
Hydroxyl radical reaction	
OH Rate constant:	5.368 x 10^{-13} cm^3/molecule-sec
Half-life:	19.927 days (12-hr/day; 1.5 x 10^{+06} OH/cm^3)
Ozone reaction:	No ozone reaction estimation
Remarks:	Estimated value based upon acceptable model
Conclusions	Material is oxidized by atmospheric hydroxyl radicals at a slow rate.
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	Estimated value based upon acceptable model.
References:	AOPWIN v1.90; Meylan, Wm. (1994). User's Guide for the Estimation Programs Interface (EPI), Syracuse Research Corporation, Syracuse, New York, 13210
Other	

B. Stability in Water

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	
Method	
Method:	Estimation
Test type:	Abiotic hydrolysis
GLP:	
Year:	
Duration:	
Remarks:	
Results	
Nominal value:	
% Degradation:	$t_{½}$ = ca. 140.6 days at pH 7, 25 °C; $t_{½}$ = ca. 14.1 days at pH 8, 25 °C
Remarks:	
Conclusions	Based on the EPIWIN results for Abiotic Hydrolysis, $t_{½}$ = ca. 140.6 days at pH 7, 25 °C; it appear that the material is not predicted to readily undergo hydrolysis.
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	Estimated value based upon acceptable model
References:	EPIWIN, v 2.0b; Meylan, Wm. & Howard, P.H.(1996). User's Guide for Estimation Programs Interface (EPI), Syracuse Research Corporation, Syracuse, New York 13210.

C. Biodegradation

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity unknown
Method	
Method:	Other; Method similar to OECD:TG-302B
Test type:	Inherent biodegradability: Modified Zahn-Wellens Test
GLP:	No
Year:	1986
Contact time:	> 5 days
Inoculum:	Mixed-liquor suspended solids from waste water treatment plant, Frankfurt./Main (Belebtschlamm, Kommunal); unacclimated
Results	
Results:	
Degradation %:	> 90% decrease in DOC (2 days) Ca. 100 % decrease in DOC (5 days)
Time for 10% degradation:	< 10 % decrease in DOC (3-hr)
Classification:	Material determined to be inherently biodegradable under the definition of the test.
Breakdown products:	Not determined
Remarks:	
Conclusions	Results indicate material would not be persistent in the environment. Test article does not require European Union labeling statement relating to long-term effects.
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	
References:	IUCLID Datensatz for Methyl acetoacetate, Submitted by Clariant GmbH, Frankfurt/Main, Germany; July 30, 1997.

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: > 99% (by GC)
Method	
Method:	Other;
Test type:	Chemical Oxygen Demand (COD)
GLP:	No
Year:	2000
Remarks:	
Results	
Results:	COD = 1.45 grams O_2 /gram of test substance
Remarks:	
Conclusions	Results indicate material would not be persistent in the environment. Test article does not require European Union labeling statement relating to long-term effects.

Data Quality **Reliability:** **Remarks:** **References:**	Reliable with restrictions Material Safety Data Sheet for Methyl acetoacetate, Eastman Chemical Company, Kingsport, Tennessee; Revision Date: November 13, 2000
Test Substance **Test Substance:** **Remarks:** **Method** **Method:** **Test type:** **GLP:** **Year:** **Remarks:** **Results** **Results:** **Remarks:** **Conclusions** **Data Quality** **Reliability:** **Remarks:** **References:**	Methyl acetoacetate Purity: > 99% (by GC) Other; Biological Oxygen Demand (BOD) No BOD_{20} = 0.77 grams O_2 /gram of test substance in 20 days; BOD_5 = 0.31 grams O_2/gram of test substance in 5 days Results indicate material would not be persistent in the environment. Test article does not require European Union labeling statement relating to long-term effects. Reliable with restrictions Material Safety Data Sheet for Methyl acetoacetate, Eastman Chemical Company, Kingsport, Tennessee; Revision Date: November 13, 2000
Test Substance **Test Substance:** **Remarks:** **Method** **Method:** **Test type:** **GLP:** **Year:** **Remarks:** **Results** **Results:** **Remarks:** **Conclusions** **Data Quality** **Reliability:** **Remarks:**	Methyl acetoacetate Purity: > 99% (by GC) Other; Total Oxygen Demand (TOD) No TOD = 1.516 grams O_2 /gram of test substance Reliable with restrictions

References:	Material Safety Data Sheet for Methyl acetoacetate, Eastman Chemical Company, Kingsport, Tennessee; Revision Date: November 13, 2000

D. Transport between Environmental Compartments (Fugacity)

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	
Method	
Test type:	Estimation
Model used:	EPIWIN Fugacity Model
Remarks:	
Results	
Model Data and results:	Water-Soil: log Koc = 0.015
Estimated distribution and media Concentration(level II/III):	Water-Air: Henry's Law Constant 2.73 x 10^{-07} atm-m^3/mol
Remarks:	
Conclusions	Material estimated to have similar distribution between water and soil. Low volatility from water to air.
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	Estimated value based upon acceptable model
References:	IUCLID Datensatz for Methyl acetoacetate, Submitted by Clariant GmbH, Frankfurt/Main, Germany; July 30, 1997.

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	
Method	
Test type:	Estimation
Model used:	Calculated according to Mackay, Level III, Syracuse Research Corporation Epiwin Version 3.05. Emissions (1000 kg/hr) to air, water, and soil compartments using standard EPA Model defaults.
Remarks:	
Results	
Model Data and results:	Air, water, soil, sediment
Estimated distribution and media Concentration(level II/III):	Air: 4.72 % Half-Life: 478-hr Emissions: 1000 kg/hr Water: 48.1 % Half-Life: 360-hr Emissions: 1000 kg/hr Soil: 47.1 % Half-Life: 360-hr Emissions: 1000 kg/hr Sediment: 0.0802 % Half-Life: 1440-hr Emissions: 0 kg/hr
Remarks:	Data used: Molecular Weight: 116.116 Vapor Pressure: 1.32 hPa @ 20°C Henry's Law Constant: 25°C (HENRYWIN v3.10) Bond Method: 1.18 x10^{-07} atm-m^3/mole Exper Database: 2.73 x10^{-07} atm-m^3/mole [VP/WSol estimate using EPI values]: 4.666 x10^{-07} atm-m^3/mole Log Kow: -0.69 (KOWWIN v1.66 estimate)

	Soil Koc: Water-Soil: log Koc = 0.015 Water-Air: Henry's Law Constant 2.73 x 10^{-07} atm-m^3/mol
Conclusions	
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	Estimated value based upon acceptable model
References:	UCLID Datensatz for Methyl acetoacetate, Submitted by Clariant GmbH, Frankfurt/Main, Germany; July 30, 1997.

IV. Ecotoxicity

A. Acute Toxicity to Fish

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: > 99%
Method	
Method:	OECD TG-203, Acute toxicity to fish
Test type:	Static
GLP:	Yes
Year:	1998
Species/strain:	Fathead Minnow(*Pimephales promelas*)
Analytical monitoring:	Yes, Exposure solution, Temperature, pH, dissolved oxygen
Exposure duration:	96-hr(also monitored at 48-hr)
Remarks:	Biological loading was kept below 1.0 g wet weight per liter of test solution, with 14 fish used per exposure level (2 replicates of 7 fish)
Results	
Nominal concentration:	125 mg/l
Measured concentration:	111.4 mg/l
Endpoint values:	96-hour LC_{50}: > 111.4 mg/l; NOEC: 111.4 mg/l
Biological observations:	No mortality observed throughout 96-hour exposure in test substance or control
Proper statistical evaluation used:	NA due to no mortality occurring
Remarks:	The determination of the LC_{50} values were based on the arithmetic average (for replicates A and B) of the geometric means of the 0-hour and 96-hour test substance analytical results. The tests were performed in glass jars containing 20 liters of exposure solution. Exposure temperature ranged from 19-20 °C, ph ranged from 7.7 to 8.4 and deoissolved oxygen ranged from 7.8 to 9.1 mg/l. The analyzed mean percent loss of the test substance ranged from 5.3% to 12.9%.
Conclusions	The 96-hour LC_{50} value indicates that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the EPA's assessment criteria.
Data Quality	
Reliability:	Reliable without restrictions
Remarks:	This is a well-documented OECD guideline study conducted under GLP assurances.
References:	An Acute Aquatic Effects Test with the Fathead Minnow (*Pimephales promelas*); Environmental ciences Section, Health and Environment Laboratories, at Eastman Kodak Company, Rochester, NY; Study No. EN-430-904102-B; Jannuary 20, 1998

B. Acute Toxicity to Aquatic Invertebrates

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: > 99 %
Method	
Method:	OECD: TG- 202 Daphnia sp. Acute Immobilization Test and Reproduction
Test type:	Static
GLP:	Yes
Year:	1997
Species/strain:	Daphid (*Daphnia Magna*)
Analytical monitoring:	Yes; Exposure solution, temperature, pH, dissolved oxygen
Exposure period:	48-hour
Remarks:	The study was conducted in general agreement with OECD test guideline 202 and European Community Annex V, Part C.2.
Results	
Nominal concentration:	250 mg/l
Endpoint value:	48-hour EC50 > 236.9 mg/l; NOEC = 236.9 mg/l
Biological observation:	The daphnids in the dilution water controls and test substance exposure solutions exhibited normal behavior and appearance throughout the test and no significant mortality was observed (≤ 10%) during the study.
Proper statistical evaluation used:	NA; No significant differences in immobility were noted between treated and control daphnids.
Remarks:	The test substance exposure concentration was based on the arithmetic average (for replicates A and B) of the geometric means of the test substance analytical results at exposure start (time 0) and the test substance analytical results at exposure end (48-hours). Exposure temperature ranged from 20-21 °C, pH ranged from 7.9 to 8.3, and dissolved oxygen ranged from 8.7 to 8.9 mg/L. The analyzed mean percent loss of the test substance ranged from 9.4% to 9.5%.
Conclusions	The EC_{50} value indicates that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.
Data Quality	Reliable without restrictions
Reliability:	This was a well-documented OECD guideline study
Remarks:	conducted under GLP assurances.
References:	An Acute Aquatic Effects Limit Test with the Daphnid (*Daphnia magna*); Environmental Sciences Section, Health and Environment Laboratories, at Eastman Kodak Company, Rochester, NY; Study No. EN-431-904102-A, October 22, 1997
Other	

C. Toxicity to Plants

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: 99.6 %
Method	
Method:	OECD: TG-201
Test type:	Alga, Growth Inhibition Test
GLP:	Yes
Year:	1997
Species/strain:	Selenastrum capriconutum
Endpoint basis:	Cell growth rate
Exposure period:	72-hour
Remarks:	
Results	
Endpoint value:	EC_{50}(24-hr) > 978.0 mg/l; NOEC(24-hr) = 310.4 mg/l EC_{50}(48-hr) > 978.0 mg/l; NOEC(48-hr) = 102.3 mg/l EC_{50}(72-hr) > 978.0 mg/l; NOEC(72-hr) = 5.4 mg/l
Remarks:	
Conclusions	The 72-hour EC_{50} value indicates that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.
Data Quality	
Reliability:	Reliable without restriction
Remarks:	This is a well-documented OECD Guideline study conducted under GLP assurances by a reputable contract laboratory.
References:	Lonza Report No. 2784. Knight, B & Reid, C.(1997). Alga, Growth Inhibition Test (72-H, EC_{50}) with P0011. (Unpublished Report). Inveresk Research International.

V. Toxicological Data

A. Acute Toxicity

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: 99.4 %
Method	
Method:	OECD TG 401
Test type:	Acute oral toxicity
GLP:	Yes
Year:	2000
Species/strain:	Rat/Crj:CD(SD)
Sex:	Males & Females
Animal/sex/dose:	5
Vehicle:	Water
Route of exposure:	Oral (gavage)
Remarks:	
Results	
Value:	LD0 = 2.0 g/kg Males; 2.0 g/kg Females LD50 = > 2.0 g/kg Males; > 2.0 g/kg Females
Deaths at each dose level:	0 g/kg(Vehicle); 0 2.0 g/kg; 0
Proper statistical evaluation used:	Yes
Remarks:	No deaths occurred in either male or female groups. No changes were observed for clinical observations, body weights or necropsy findings.
Conclusions	Material, based upon results, was not considered to be toxic.
Data Quality	
Reliability:	Reliable without restriction.
Remarks:	This was a well-documented OECD guideline study conducted under GLP assurances conducted by Safety Assessment Laboratory, Panapharm Laboratories Co., Ltd.,Kumamoto, Japan.
References:	Japan Chemical Industry Ecology-Toxicology & Information Center, JETCO Information Sheet No. 43, Methyl acetoacetate, April 2000

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: ≥ 99 % (by GC)
Method	
Method:	Other; FHSA
Test type:	Acute oral toxicity
GLP:	No
Year:	1975
Species/strain:	Rat
Sex:	Males & Females
Animal/sex/dose:	5

Vehicle:	None indicated
Route of exposure:	Oral
Remarks:	
Results	
Value:	LD_0 = 2.0 g/kg Males; 2.5 g/kg Females LD_{50} = 2.58 g/kg (95 % C.L. 2.23 – 2.98 g/kg) Males LD_{50} = 3.37 g/kg (95 % C.L. 2.92 – 3.90 g/kg) Females LD_{100} = 4.0 g/kg Males; 8.0 g/kg Females
Deaths at each dose level:	1.0 g/kg; 0 2.0 g/kg; 0 2.5 g/kg; 3 males (Day 1) 3.2 g/kg; 4 males (Day 1), 2 females (Day 1) 4.0 g/kg; 5 males (Day 1 and 3), 4 females (Day 1) 8.0 g/kg; 5 males (Day1), 5 females (Day 1)
Proper statistical evaluation used:	Yes
Remarks:	Animals dosed at 1.0- or 2.0 g/kg were observed to be sluggish and unkempt following intubation. Within 48-hours animals returned to normal behavior and appearance. Animals dosed at 2.5- to 4.0 g/kg exhibited lethargy, nasal hemorrhage and dirty, unkempt coats. At 8.0 g/kg all animals became comatose immediately following intubation and succumbed within 15 minutes of exposure. No postmortem or histopathology examinations were performed on any animals.
Conclusions	Material, based upon results, is considered to be slightly toxic.
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	This study is a well-documented study.
References:	Lonza Report No. 0113 Acute oral toxicity to rats of P0011. (Unpublished Report).Bio-Toxicology Laboratories, Inc., Moorestown, NJ.

B. Repeated Dose Toxicity

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: 99.7 %
Method	
Method:	Other; Modified OECD 407
Test type:	4 Week toxicity study with 2 week recovery period .
GLP:	Yes
Year:	1996
Species/strain:	Rat/Sprague-Dawley (Crl:CD® BR)
Route of exposure:	Daily oral gavage
Duration of test:	4-Week exposure with 2-week recovery for control and high dose subgroups.
Dose levels:	0, 100-, 300- and 1000 mg/kg-day
Sex:	Both
Control group & treatment:	Controls received vehicle (water)
Post-exposure observation period:	2-Week recovery for control and high dose subgroup.
Remarks:	This study was designed to be compliant with the guidelines of the European Union and Japanese MITI.
Results	
NOAEL:	1000 mg/kg-day
Toxic responses by dose::	**Observations**:There were no premature deaths during the study. No clinical signs observed were considered to be as a result of treatment with the test substance. There were no notable intergroup body weight, food or water consumption differences in either sex. **Laboratory Investigations**: There were no effects noted in hematology, clinical chemistries or urinalyses considered to be toxicologically significant in either sex. **Terminal Studies**: There were no intergroup differences in either organ weights, necropsy or histological findings that were considered to be treatment related in either sex. On completion of 4 weeks of dosing all main study animals were sacrificed and necropsied and given a detailed post mortem examination with organs being weighed (i.e. adrenals, brain, kidney, liver, ovaries and testis with epididymis) and tissues preserved in fixative (i.e. all abnormnal tissue, adrenals, brain, ears, eyes, heart, kidney, liver, lung, marrow smear, optic nerve, ovaries, pituitary, spleen stomach, testis with epididymis, thyroid with parathyroid and urinary bladder). Histologolical examination was carried out on all control and high dose animals. After the 2 week recovery period all recovery study animals werr sacrificed and processed in an identical manner
Proper statistical evaluation used:	Yes
Remarks:	Body weight, food consumption, hematology, clinical chemistry, urinalysis and organ weight data were statistically analyzed for homogeneity of variance using the 'F-max' test. If the group variance appeared homogeneous a parametric ANOVA was used and pairwise comparisons made *via* Student t-test using Fisher's F-protected LSD. If the variances were heterogeneous, log or square root transformations were used in an attempt to stabilize the

	variances. If variances remained heterogeneous, then a nonparametric test such as Kruskal-Wallis ANOVA was used. Organ weights were also analyzed conditional on body weight *i.e.* analysis of covariance(ANCOVA). Histology data were analyzed by Fisher's Exact Probability test.
Conclusions	Material induced minimal toxicity following 4-weeks of exposure.
Data Quality **Reliability:** **Remarks:**	Reliable without restriction. This is a well-documented study conducted in compliance with EU OECD and Japanese MITI guidelines under GLP assurance.
References:	Lonza Report No. 2500. 4-Week Toxicity Study in Rats with Administration by Gavage and with 2-Week Recovery Period

C. Genetic Toxicity - Mutation

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: > 99 %
Method	
Method:	OECD: TG-471; Bacterial Reverse Mutation Test
GLP:	Yes
Year:	1988
Species/strain:	*Salmonella typhimurim* TA1535, TA1537, TA1538, TA98 & TA100
Metabolic activation:	Yes
Concentration tested:	0 to 5000 µg/plate (5 strains)
Remarks:	The study was conducted in triplicate after first determining the maximum concentration that does not significantly modify bacterial growth using the TA98 and TA100 test strains.
Results	
Results:	Material did not induce gene mutation in any of the *Salmonella typhimurim* strains tested.
Cytotoxic concentration::	➢ 5000 µg/plate
Precipitation concentration:	No precipitate was reported at the maximum concentration tested.
Genotoxic effects	
With activation:	Negative
Without activation:	Negative
Proper statistical evaluation used:	Yes
Remarks:	
Conclusions	Material was determined not to be mutagenic under the conditions of the assay.
Data Quality	
Reliability:	Reliable without restrictions.
Remarks:	This was a well-documented OECD guideline study conducted under GLP assurances conducted by Hoechst AG, Pharma Forschung Toxikologie, Frankfurt, Germany.
References:	Lonza Report No. 0117. Reverse Mutation Assay In Vitro Ames Test with P0011. (Unpublished Report). Hoechst AG, Pharma Forschung Toxikologie, Report No. 3848 MMO.
Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: 99.4 %
Method	
Method:	Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD 471 and 472
GLP:	Yes
Year:	1996
Species/strain:	*Salmonella typhimurim* TA100, TA1535, TA98, TA1537, *Escherichia coli* WP2 *uvr*A
Metabolic activation:	Yes
Concentration tested:	0, 313 to 5000 µg/plate(5 strains)

Remarks:	The study was conducted in duplicate in the *Salmonella typhimurim* strains and in triplicate in the *Escherichia coli* WP2 *uvr*A.
Results	
Results:	Material did not induce gene mutation in any of the *Salmonella typhimurim* or *Escherichia coli* WP2 *uvr*A strains tested.
Cytotoxic concentration::	➢ 5000 µg/plate
Genotoxic effects	
With activation:	Negative
Without activation:	Negative
Proper statistical evaluation used:	Yes
Remarks:	
Conclusions	Material was determined not to be mutagenic under the conditions of the assay.
Data Quality	
Reliability:	Reliable without restrictions.
Remarks:	This was a well-documented OECD guideline study conducted under GLP assurances conducted by Hatano Research Institute, Food and Drug Safety Center, Kanagawa, Japan.
References:	Japan Chemical Industry Ecology-Toxicology & Information Center, JETCO Information Sheet No. 43, Methyl acetoacetate, April 2000

D. Genetic Toxicity – Chromosomal Aberrations

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: 99.4%
Method	
Method:	Guideline for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guideline No. 473
GLP:	Yes
Year:	1996
Species/strain:	Chinese hamster lung (CHL/IU) cells
Metabolic activation:	Yes, Phenobarbital & 5,6-benzoflavone induced rat liver S9.
Concentration tested:	0(solvent), 0.30, 0.60 and 1.2 mg/ml
Test conditions:	Solvent: Distilled water Positive controls: -S9 mix, Mitomycin C +S9 mix, Cyclophosphamide Test exposures: -S9 mix (continuous treatment) -S9 mix (Short –term treatment) +S9 mix (Short –term treatment) +S9 mix (Short –term treatment with pH-adjustment): 0, 1.2 mg/ml
Remarks:	Each test was conducted in duplicate.
Results	
Results:	Material caused a significant increase in structural chromosomal aberrations (including gaps) and polyploidy following short-term treatment with metabolic activation, +S9 mix, at 1.2 mg/ml.
Cytotoxic concentration:	1.2 mg/ml
Genotoxic effects	
With activation:	Positive with short-term treatment
Without activation:	Negative
Proper statistical evaluation used:	Yes
Remarks:	Addition of Methyl acetoacetate at 1.2 mg/ml significantly decreased the pH of the medium. To evaluate the effect of pH reduction, a confirmatory test was conducted where the pH of the medium was adjusted. After pH adjustment, no significant increase in structural chromosomal aberrations or polyploidy were observed.
Conclusions	No genotoxic effects were observed at any of the exposure concentrations tested without metabolic activation. Equivocal genotoxic effects were observed at an exposure concentration of 1.2 mg/ml following short-term treatment with exogenous metabolic activation. It has been suggested that the chromosomal aberrations induced were caused by the lowering of the pH of the medium by the addition of the Methyl acetoacetate and not by the substance itself.
Data Quality	
Reliability:	Reliable without restrictions.
Remarks:	This was a well-documented OECD guideline study conducted under GLP assurances conducted by Hatano Research Institute, Food and Drug Safety Center, Kanagawa, Japan.

References:	Japan Chemical Industry Ecology-Toxicology & Information Center, JETCO Information Sheet No. 43, Methyl acetoacetate, April 2000

E. Repeated Dose and Reproductive/Developmental Toxicity

Test Substance	
Test Substance:	Methyl acetoacetate
Remarks:	Purity: 99.4 %
Method	
Method:	OECD TG-422 Combined Repeat Dose and Reproductive/ Developmental Toxicity Screening Test
Test type:	Repeated Dose and Reproductive/Developmental Toxicity Study in Rats with Administration by Gavage.
GLP:	Yes
Year:	1996
Species/strain:	Rat/ Crj: CD(SD)
Sex:	Both
Number of animals/group:	12/sex
Route of exposure:	Oral gavage
Dose levels:	0(Vehicle), 100-, 300- and 1000 mg/kg-day
Exposure period:	Males were treated for 7 weeks; females were treated from 14-days prior to mating through day-3 of lactation.
Frequency of treatment:	Daily
Control group treatment:	Controls received vehicle (water)
Remarks:	This study was designed to be compliant with the guidelines of the European Union and Japanese MITI.
Results	
Repeated Dose	
Toxicity, NOEL:	>1000 mg/kg-day
Toxic responses by dose::	**Observations**:There were no premature deaths during the study. No clinical signs observed were considered to be as a result of treatment with the test substance. There were no notable intergroup body weight, food or water consumption differences in either sex. **Laboratory Investigations**: There were no effects noted in hemotology or clinical chemistries considered to be toxicologically significant in either sex. **Terminal Studies**: There were no intergroup differences in either organ weights, necropsy or histological findings that were considered to be treatment related in either sex.
Reproductive/Developmental	
Parental Toxicity, NOEL:	>1000 mg/kg-day
Paternal/maternal toxic responses by dose:	**Reproductive Performance:** There were no treatment related effects noted in estrous cycle, number of corpora lutea and implantations, copulation or fertility indices for males or females.
Fetal Toxicity, NOEL:	>1000 mg/kg-day
Fetal toxic responses by dose:	**Development:** There were no treatment related effects noted on gestational days, litter and live born numbers,

	gestation index, stillborn index, birth index, sex ratio, body weights of offspring at birth and at day 4 post birth, or viability index on day 4. No external abnormalities were observed.
Proper statistical evaluation used:	yes
Remarks:	
Conclusions	Material was determined not to induce reproductive or developmental toxicity under the conditions of the assay.
Data Quality	
Reliability:	Reliable without restriction.
Remarks:	This was a well-documented OECD guideline study conducted under GLP assurances conducted by Safety Assessment Laboratory, Panapharm Laboratories Co., Ltd.,Kumamoto, Japan.
References:	Japan Chemical Industry Ecology-Toxicology & Information Center, JETCO Information Sheet No. 43, Methyl acetoacetate, April 2000